

# **Deep Focus 3.5**

## **User Guide**

# Deep Focus 3.5

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# Preface

The Deep Focus 3.5 is an additional extension software module for the QuickPHOTO programs, that creates completely in-focus images, which cannot be achieved by a standard use of microscopes, because of their limited depth of field. This technique is called EDF - Extended Depth of Field imaging, focus stacking or z-stacking. The Deep Focus 3.5 module can be used in conjunction with stereomicroscopes as well as with other types of optical microscopes for observation in transmitted or reflected light. The module is also suitable for macro-imaging.

## Principle of Function

The completely in-focus image is composed from several standard images acquired at different focus levels - so called "slices". From each of the slices, only the in-focus areas are used. The completely in-focus image is composed from these in-focus areas. Possible shifts and scale changes between slices are compensated automatically.



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# Installation

## Note

For a successful Deep Focus 3.5 module installation, it is necessary to have the *QuickPHOTO CAMERA*, *QuickPHOTO MICRO* or *QuickPHOTO INDUSTRIAL* program in the 3.1 or higher version (the newest version is recommended) installed.

## Note

In case you do not have the permission to use the Deep Focus 3.5 module contained in your dongle or you are installing the module into a trial version of the one of the above mentioned programs (no dongle is used), the Deep Focus 3.5 module will run in a demo version mode. The demo version provides all the functions of a full version, but a watermark will be inserted into the created images. Each time you run the demo version you will be informed about that fact. If you want to purchase the full version, contact your software supplier.

To install the Deep Focus 3.5 module from the supplied installation disk:

1. Make sure the *QuickPHOTO* program is not running. If it is running, terminate it.
2. Insert the installation disk into the DVD drive. The autorun screen appears.
3. If the autorun screen does not appear, run the *autorun.exe* file from the installation disk.
4. Select *Install Deep Focus 3.5*.

To install the Deep Focus 3.5 module from the downloaded installation package:

1. Make sure the *QuickPHOTO* program is not running. If it is running, terminate it.
2. Run the installation package.
3. Select the destination folder, where the installation files should be extracted.
4. Wait until the installation files are extracted and the autorun screen appears.

The following procedure is the same for both above mentioned variants of installation. The installation wizard appears. Perform the following steps:

1. Select the desired language.
2. Click the *OK* button and continue with the *Next* button. The installation wizard searches for the installed *QuickPHOTO CAMERA*, *QuickPHOTO MICRO* and *QuickPHOTO INDUSTRIAL* programs.
3. If there are multiple versions of the QuickPHOTO program installed, select the desired version for Deep Focus 3.5 module installation and click the *Next* button.
4. If your use a microscope focus motorization unit (a focus drive or a motorized stand) controlled by the CB-ZM control unit, keep the *Install USB Serial Port Device Drivers* option checked.
5. Click the *Next* button. Continue following the on-screen instructions.

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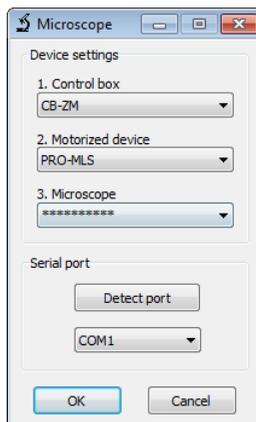
# Settings for Motorized Image Capturing

The process of creating the completely in-focus images can be automated by the use of an optional focus drive or a motorized stand. To automate the process of image capturing, the Deep Focus 3.5 module cooperates with a software module for motorized focus control. This module called *Microscope* is installed together with the Deep Focus 3.5 module and can also be run separately by clicking the  icon in the *Toolbar* of the QuickPHOTO program.

The *Microscope* module supports several types of control units, motorized focus drives, stands and motorized microscopes. This module needs to be configured prior to its first use.

In order to set the motorized focus control proceed as follows:

1. Make sure the focusing unit is properly installed and connected to its control unit. Further, make sure the control unit is switched on, connected to the computer and its device drivers are properly installed.
2. In the QuickPHOTO program activate the *Modules* → *Microscope* function or click the  icon in the *Toolbar*.
3. In the opened *Microscope* dialog box in the *Device settings* part, select a desired control unit first, from the *1. Control box* drop-down menu. Then select a motorized device from the *2. Motorized device* drop-down menu, and finally select a microscope type from the *3. Microscope* drop-down menu.



**Note**

To achieve a proper z-axis calibration, it is important to select a proper microscope type. Z-axis calibration of some motorized devices is not dependent on a microscope type - for such devices, there will appear "\*\*\*\*\*" option in 3. *Microscope* drop-down menu.

4. In the *Serial port* part select the COM port the control unit is connected to. Use the *Detect port* button to find out the correct port.
5. Click the *OK* button to confirm the settings.
6. The *Microscope* module will switch to the operational mode. Use the *Up* and *Down* buttons to test its functionality.
7. If the *Microscope* module properly controls the motorized device, close it.
8. If, when the *Up* button is pressed, the microscope focuses in an opposite direction, mount the focus drive to the opposite side of the microscope focus knob, or change the internal settings of the CB-ZM control unit using a software tool delivered with it. For more information refer to a user guide of the CB-ZM control unit.

After completing this procedure, you can start using the functions for motorized image capturing of the Deep Focus 3.5 module.

If necessary, the *Microscope* module can be switched to the configuration mode at any time by clicking the *Device settings* button.

**Note**

The CB-ZM control unit is connectible to the computer via a USB cable, however it communicates with a PC in the manner of a serial port (COM). This device includes a special converter and there will be installed a USB serial port in the operating system. The CB-ZM device is usually installed on the COM port with the highest number.

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# Manual Capturing of Slices for Composition

The first step to create a completely in-focus image is capturing of a series of images acquired at different focus levels - so called "slices". In each slice a different area of the sample should be in-focus. Use a microscope focus knob to change the focus level. The focus level change between subsequent slices should not be greater than is a depth of field of the microscope. It means the in-focus area should move smoothly over the sample in the series of the captured slices.

## Recommended Procedure for Capturing Slices

1. Place the sample under the microscope and set the illumination.
2. In the QuickPHOTO program, open the control panel of your imaging device.
3. In the imaging device control panel set the manual exposure mode and set the desired exposure time. If the automatic exposure mode is preferred, activate the *Auto Exposure Lock (AEL)* function, to avoid exposure changes during image capturing.
4. Using the live view, focus the microscope to the very bottom part of the sample.
5. Acquire the first image.
6. Change the microscope focus level slightly upwards. Acquire the second image.
7. Similarly acquire other images, until the highest part of the sample is acquired. Always make sure, the slices are acquired in correct focus order.

### Note

It is also possible to proceed in reverse: first focus on the highest part of the sample and gradually refocus downwards.

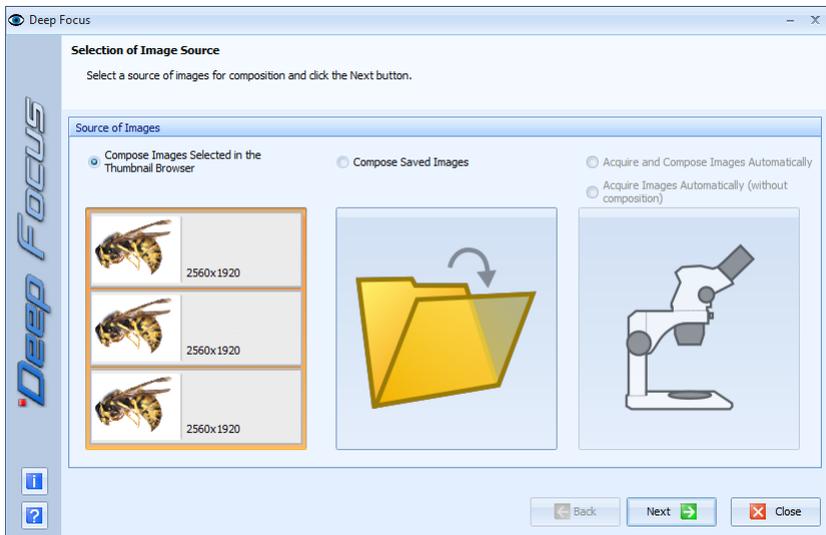


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# How to Use Deep Focus 3.5 Module

The Deep Focus 3.5 module can be used in following ways:

1. Compose images selected in the *Thumbnail Browser*
2. Compose saved images
3. Acquire and compose images automatically
4. Acquire images automatically (without composition)



## 1. Compose Images Selected in the *Thumbnail Browser*

This option is intended for composition of images opened in the QuickPHOTO program.

The procedure of use is following:

1. Select the images (intended for composition) opened in the QuickPHOTO program by selecting their thumbnails in the *Thumbnail Browser*. The selected images will be indicated by frames. The selection can be performed similarly to the selection of files in Windows® Explorer (all images can be selected by [Ctrl+A] key shortcut, the continuous selection can be done while holding the [Shift] key or respectively [Ctrl] key for discontinuous selection). Make sure the selection does not contain any unwanted images.
2. Launch the Deep Focus 3.5 module from *Modules* → *Deep Focus 3.5* menu or by clicking the  icon.
3. In the opened dialog box select the *Compose Images Selected in the Thumbnail Browser* option and click the *Next* button.
4. The next page of the wizard *Composition Parameters* will display. To continue see “Setting the Parameters of Composition and In-focus Image Composition“ on page 18.

**Important**

All images to be processed must have the same dimensions in pixels (e. g. 2448 x 2048).

## 2. Compose Saved Images

This option is intended for composition of images stored on your hard drive, flash memory drive etc.

The procedure of use is following:

1. Launch the Deep Focus 3.5 module from *Modules* → *Deep Focus 3.5* menu or by clicking the  icon.
2. In the opened dialog box select the *Compose Saved Images* option and click the *Next* button.
3. Use the *Open* dialog to select the images to be composed. Make sure the selection does not contain any unwanted images.

4. The next page of the wizard *Composition Parameters* will display. To continue see “Setting the Parameters of Composition and In-focus Image Composition“ on page 18.

**Important**

All images to be processed must have the same dimensions in pixels (e. g. 2448 x 2048).

### **3. Acquire and Compose Images Automatically Using the Directly Supported Imaging Devices**

This option is intended for automated acquisition and composition of completely in-focus images using the imaging devices directly supported by the QuickPHOTO programs and microscope focus motorization (focus drives or motorized stands).

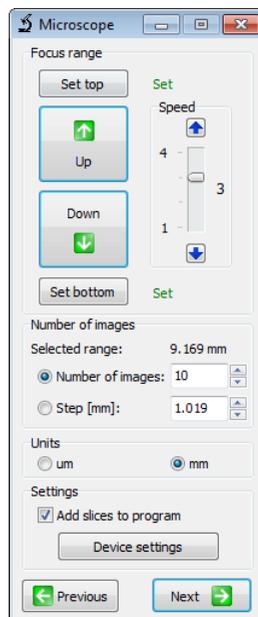
The procedure of use is following:

1. Make sure the control unit of the focus drive or a motorized stand is switched on.
2. Place the sample under the microscope and set the illumination.
3. Launch the Deep Focus 3.5 module from *Modules* → *Deep Focus 3.5* menu or by clicking the  icon.
4. In the opened dialog box select the *Acquire and Compose Images Automatically* option and click the *Next* button.
5. The control panel of active imaging device will open along with the *Microscope* module for focus control.
6. In the imaging device control panel, set the manual exposure mode and set the desired exposure time. If the automatic exposure mode is preferred, activate the *Auto Exposure Lock (AEL)* function, to avoid exposure changes during image capturing.

7. Set the focus range using the *Microscope* dialog box as follows:

- a. Use the *Up* or *Down* buttons to focus (using the live view) the microscope to the very bottom part of the sample. Click the *Set bottom* button to set the lower limit of the focus range. The *Not set* text on right side of the *Set bottom* button will change to *Set*.
- b. Use the *Up* button to focus (using the live view) the microscope to the highest part of the sample. Click the *Set top* button to set the upper limit of the focus range. The *Not set* text on right side of the *Set top* button will change to *Set*.

The focusing can be also controlled by the keyboard (using the up and down arrow keys). Focusing speed can be set by the *Speed* slider or also by the [+] or [-] keys.



### Note

It is also possible to proceed in reverse: first focus on the highest part of the sample and then to its lowest part.

8. In *Number of images* part enter the desired *Number of images* (slices) to be acquired or enter the *Step* (distance) between slices. The corresponding second parameter will be calculated automatically. The *Selected range* indicator shows distance between lower and upper limit of the set focus range in units selected in *Units* part.
9. If you want to keep the slices, from which the image is going to be composed, check the *Add slices to program* checkbox. The slices will appear in the QuickPHOTO program after acquisition.

10. After clicking the *Next* button in the *Microscope* dialog box there will appear the *Composition Parameters* page of the wizard. To continue see “Setting the Parameters of Composition and In-focus Image Composition“ on page 18.

**Important**

In order to begin the process of automated capturing of slices and image composition, it is necessary to set the lower and upper limit of the focus range and the number of slices to be acquired or a step between them.

In case the *Step* between slices is selected, the selected focus range will be divided into integral multiple of the selected step size and the remaining rest of the range, e.g. focus range is set to 170 microns, the selected step is 50 microns - the focus range will be divided into 3 steps of 50 microns and one step of the remaining 20 microns.

The refocusing during the image acquisition process will be performed with the speed set by the *Speed* slider in the *Microscope* dialog box. That focusing speed should not be set to high, to maintain focusing precision and to avoid vibrations.

## **4. Acquire and Compose Images Automatically Using the Imaging Devices Controlled via TWAIN Interface**

Deep Focus 3.5 module enables automated image capturing and composition also from imaging devices controlled via TWAIN interface. Such imaging devices are controlled by the Deep Focus 3.5 module externally, which implies minor limitations.

The procedure is similar to the one described in the previous chapter, but after you start the image acquisition (by clicking the *Start* button) using the TWAIN control panel, an informative dialog box will be displayed. You will be asked to click the button for image capturing in the control panel of the imaging device. After you click the corresponding button, initialization image will be captured (which will not be included in the composition), which will enable the Deep Focus 3.5 module to identify the position of the button for image capturing in the TWAIN control panel. Information dialog box will automatically disappear after capturing of this image and the image acquisition process will begin. To

ensure successful process of image acquisition, do not move or click the mouse anymore until the whole image acquisition process is finished.

## **5. Acquire Images Automatically without Composition**

This option is intended for automatic acquisition of slices without composition. The slices will be displayed in the QuickPHOTO program immediately after their acquisition and the process of composition will be omitted. The procedure of use is similar to the articles 3 and 4.

## **6. Setting the Parameters of Composition and In-focus Image Composition**

In this step set the composition parameters and start the composition process as follows:

1. On the *Composition Parameters* page, select the type of images to be composed.

There are two options available:

- **Aligned Images**

### **Images from Upright and Inverted Microscopes**

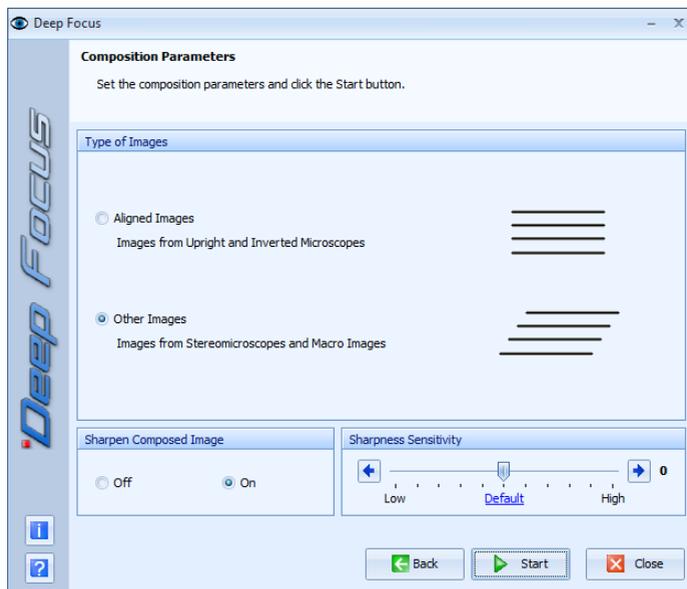
Select this option for composition of images acquired from a microscope which does not cause shifting of the sample in the field of the imaging device's view during refocusing (upright and inverted microscopes). Composition of aligned images is faster than the composition of shifted images (images from stereomicroscopes).

- **Other Images**

### **Images from Stereomicroscopes and Macro Images**

Select this option for composition of images acquired from stereomicroscopes, macrosopes and for macro images. Stereomicroscopes generally cause shifting of the sample in the field of the imaging device's view while refocusing. Within macro images the minor changes of a scale (magnification) usually take place. The Deep

Focus 3.5 module compensates the shifts and scale changes between images automatically in the *Other Images* mode.



2. In the *Sharpen Composed Image* part select, whether you want to post-process the composed image with a sharpen operation. This operation enhances edges and fine details in the composed image.
3. Leave the *Sharpness Sensitivity* slider in the *Default* position for most cases. In case the composed image contains some unfocused parts, although they are in-focus in some of the slices, increase the *Sharpness Sensitivity* value and repeat the composition process. The higher sensitivity you set, the more significant presence of noise, which is present in all digital images, can be. The noise level depends mainly on quality, type and settings of the used imaging device as well as on illumination conditions. For example, when using a cooled digital camera, the noise level will be rather low. Conversely, when using a consumer digital camera with high ISO sensitivity set in

poor illumination conditions, the noise level will be quite significant. The noise impact, when the *Sharpness Sensitivity* value is set too high, is usually manifested by a presence of many unsharp areas (islands) in the composed image. In some cases decrement of *Sharpness Sensitivity* value may also be required.

4. Click the *Start* button to begin the composition process. The *Start* button will change to *Stop* button, which can be used to interrupt the process of composition. The composed image will be displayed in the main program window after finishing the composition process. It will be named *f\_#####.suffix*, where ##### is a serial number assigned to the image by the QuickPHOTO program. If necessary, you can interrupt the composition process and close the Deep Focus 3.5 dialog box by clicking the *Close* button.

**Note**

The dimensions of a composed image will be slightly smaller than are the dimensions of the slices, due to an automatic crop function.

**Note**

The composition speed depends on the number of slices, their resolution and power of the computer.

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# Creating Completely In-Focus HDR Images in Conjunction with Optional HDR Module

The Deep Focus 3.5 module can be used in conjunction with optional HDR module for creating completely in-focus HDR images.

## 1. Manual Acquisition of HDR Slices and Their Composition to a Completely In-Focus HDR Image

The following procedure can be used for all imaging devices supported by the QuickPHOTO programs:

1. Acquire, with use of the HDR module (according to the instructions in the user guide of the HDR module), particular HDR slices - see “*Manual Capturing of Slices for Composition*“ on page 11, with the only difference, that each of the slices will be an HDR image merged by the HDR module from a series of images with different exposure times.
2. Compose the acquired HDR slices by the Deep Focus 3.5 module into a completely in-focus HDR image.

## 2. Automated Creation of Completely In-Focus HDR Images

Creation of completely in-focus HDR images can be automated when using PROMICAM USB 3.0, PROMICAM LITE or Canon cameras and a microscope focus motorization (focus drives or motorized stands).

The procedure of completely in-focus HDR image creation is similar to the procedure of the automated creation of standard completely in-focus images - see “Acquire and Compose Images Automatically Using the Directly Supported Imaging Devices“ on page 15 with

one difference. Before starting the process of automated images acquisition, activate and set the HDR feature in the control panel of the imaging device (according to the instructions in the user guide of the HDR module). After starting the process, a series of HDR slices will be automatically acquired and subsequently composed to a completely in-focus HDR image by the Deep Focus 3.5 module.

**Note**

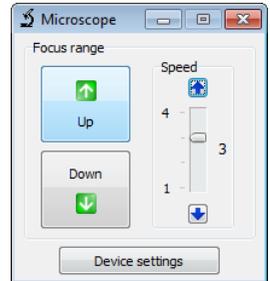
The automated creation of completely in-focus HDR images is not available for imaging devices controlled via TWAIN interface.

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# Independent PC Control of a Motorized Focusing

After installation of the Deep Focus 3.5 module, you can control the motorized focusing of the microscope from the QuickPHOTO program also without launching the Deep Focus 3.5 module. For the focus control you can use the *Microscope* software module, which can be launched by clicking the  icon in the QuickPHOTO program *Toolbar*.

Using the *Down* button you can focus to the lower parts of the sample. Using the *Up* button you can focus to the higher parts of the sample. Focusing can be also controlled by the keyboard, using the up and down arrow keys. Speed of the focus movement can be adjusted by the *Speed* slider or also by the [+] or [-] keys.





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# Tips for Work with Deep Focus 3.5

Here there are some recommendations to achieve the best results with Deep Focus 3.5 module:

1. Place the specimen to the centre of the imaging device's field of view and fix it, to avoid its movements or rotation during image capturing.
2. Capture the whole series of slices with the same settings of the imaging device and the microscope. Do not change the zoom value during capturing of slices.
3. Use manual exposure mode for acquisition of slices. If the automatic exposure mode is preferred, activate the *Auto Exposure Lock (AEL)* function, to avoid exposure changes during image capturing.
4. Do not change the illumination conditions during the capturing of slices.
5. Use a *Shading Correction* feature (if your imaging device provides it) to achieve the best illumination homogeneity. For more information about this feature, refer to the user guide of the *QuickPHOTO* program.
6. Make sure, the slices are acquired in a correct focus order. If the order is incorrect, the resulting image will be probably composed incorrectly.
7. The best results can be achieved, when capturing higher number of slices with small changes of the focus level.
8. If some areas in the resulting image are unsharp, reduce the focus step size between the slices.
9. Make sure, there is no unwanted image in your selection of images intended for composition.
10. Maximal resolution of slices, which can be composed by the Deep Focus 3.5 module in 32bit Microsoft® Windows® operating systems is approx. 22MP. For composition of slices with higher resolution, use a 64bit version of operating system and enough RAM.

11. To achieve the highest speed of composition, use a computer with the most powerful processor with as many cores as possible.

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# Measurements in Composed Images

It is possible to perform measurements also in the composed images. However, the measurement should be considered as indicative, as the measurement error in composed images can reach up to 5% compared to the measurement in input images (slices).

To activate the measuring tools, it is necessary to first assign a calibration to the composed image, ie. choose from the *Objective* and *Zoom* menus in the toolbar the magnification, with which the input images (slices) were acquired. For more information on measurement options, see the QuickPHOTO program user guide.

The Deep Focus 3.5 module automatically assigns a calibration to a composed image when it is created, if any of the slices contain calibration information.



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# System Requirements

## Minimal Computer Configuration:

- Dual-core processor
- 2 GB RAM
- Microsoft® Windows® 7/8.1/10 (32bit / 64bit)\*

## Recommended Computer Configuration:

- Intel® Core i5 / AMD RYZEN 5 processor or better (The more cores the processor has, the higher the composition speed is).
- 4 GB RAM or more
- Microsoft® Windows® 10 64bit\*

\* When using Microsoft® Windows® edition "N", you may need to download and install the *Media Feature Pack* from Microsoft's website.

## Supported QuickPHOTO Program Versions:

- QuickPHOTO CAMERA 3.1 and newer (the latest version is recommended)
- QuickPHOTO MICRO 3.1 and newer (the latest version is recommended)
- QuickPHOTO INDUSTRIAL 3.1 and newer (the latest version is recommended)

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